

# Observation on Ultra-micro Structure of Eggshell and Analysis of Composition of Eggshell and Feather in Great Bustard

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**Abstract** Ultra-micro structure of eggshell was observed and compositions of eggshell and feather were analyzed. The results showed that the structure of eggshell has special characters besides common structure. The content of poisonous elements in wild great bustard (*Otis tarda*) eggshell and feather is significant lower than that in eggshell of crested ibis (*Nipponia nippon*) and in feather of red crown crane (*Grus japonensis*) because of less disturbance by people. The contents of 14 elements of female's feather all are higher than those of male's.

**Key word:** Great bustard *Otis tarda*, Eggshell, Feather, Ultra-micro structure, Content of element

## Introduction

Great Bustard (*Otis tarda*), Gruiformes, Otidae. It is mainly distributed in Europe, south of Siberia, and Inner Mongolia Autonomous region, Jilin Province and Heilongjiang Province in China. It is near extinction because of overhunting, therefore, it was labeled in Appendix I of CITES in 1987. In China, it was raised artificially since the early of 1950s, but first tried in zoos in 1987. Up to now, there are still many technical problems on artificial breeding being unsolved. So research on great bustard will provide scientific basis for artificial breeding and be an important countermeasure to recover the population. Previous researches mainly centered on ecology, behavior artificial raising, artificial incubation and body temperature dynamics of fledgling, etc.. No reports have been found on the ultra-microstructure of eggshell and composition analysis of eggshell and feather. The observation can provide taxological and evolution information. The analysis can be of great significance with respect to raising management, prevention, treatment and diagnosis of diseases.

## Materials and Methods

### Sampling

Eggshell were collected after nestings were just hatched in Xing'an League grassland in Inner Mongolia. Feathers were sampled from the chests of wild mature great bustard which were just moved in Harbin Zoo.

### Preparation for scanning electron microscopic

A 25 mm<sup>2</sup> eggshell specimen of great bustard was

taken between obtuse end and equator of egg. It was cleaned, fixed, washed, dehydrated and dried. (The details were explained by Lu Taichun 1992) Then, it was examined by S-520 scanning electron microscope

### Preparation for elements analysis of eggshell

A specimen was dried at 105~110°C in oven, pestled into pieces of 200 μm<sup>3</sup>. 0.5 g polytetrafluoroethylene was weighted and put into crucible (50 mL), mixed with hydrofluoric acid 7-8 mL, 1 mL HClO<sub>3</sub> and 1 mL HNO<sub>3</sub>, and then evaporated at 300°C. After sample got cool, 1:1 HCl was added to be totally 50 mL. The solution was analyzed with WFX-1C atomic absorption apparatus.

### Preparation for element analysis of feathers

The feathers were cleaned and dried at 105~110°C in oven, then, sheared into small pieces. 0.5 g feathers were weighted for the procedure as mentioned above.

### Analysis method

In the analysis of 14 elements, capacity method was adopted for analyzing Ca, calorimetric method for atomic absorption spectrophotometry for Mg, Na, Mn, Fe, Cu and Zn, atomic absorption assay in Z-700 flame-free graphite furnace for Sr, Co, Pb and Hg, 0.5g eggshell sample was put into a 25 mL calorimetric cylinder, then 20 mL 1:1 nitrohydrochloric acid was added in. The sample was heated at 200 °C to be totally dissolved 10 mL solution was used after adding potassium borohydride and ascorbic acid to be analyzed with AFS-120 atomic fluorescence analyzer.

## Result and Discussion

### Observation of eggshell structure

Cross section and outside surface of eggshell, inner membrane, outer membrane and inner surface of inner membrane air cavity were observed, respectively by scanning electron microscope.

**Cross section of eggshell** By scanning electron microscope, we can commonly divide the eggshell into three layers: papillar layer, railings layer, Crystal layers(Fig. 1a). The papillar is hollow(Fig. 1b), a papillar cove is surrounded with calcium crystals, that are emitting from the junction of railings and outer membrane, and fuses at papillar base. Cross section of papillar is cone-shaped that is arranged like flower valve (Fig. 1c). Between papillar, there are obvious cavities. The junction of papillar layer and outer membrane is so formed that papillar cove is surrounded by outer membrane fibers. There is no significant boundary between papillar and railings layer. Both of them consist of calcium crystals. Outside the railings layer, there is Crystal layer that is much finer and smoother than railings layer. This crystal layer has protective functions. According to the authors' assume that its protective functions should be based on the slick surface and buffer effects of mechanical forces. This can be proved by a lot of striae on the outer surface of eggshell (Fig. 1d). No crevasse but striae and spiracle are seen in the eggshell surface. We found that it is spiracle that causes the egg surface undulated.

The thickness of eggshell (from outside surface to the bottom of papillar) is 390  $\mu\text{m}$ , among which, crystal layer is about 10  $\mu\text{m}$ , railings layer about 300  $\mu\text{m}$  and papillar layers about 80  $\mu\text{m}$ .

**Eggshell membrane** Eggshell membrane can be divided into inner membrane(Fig. 1e) and outer membrane(Fig. 1f) which consist of thick and slim fibers. The fibers go in multi-directions but paralleled to the surface of eggshell to form a very delicate network. There are pellet-shaped matters with a diameter of 3  $\mu\text{m}$  or so on the fibers of outer and inner membrane. They may be microbe parasiting on shell membrane. But some scholars considered them as sprout inflation(Li Fulai *et al.*, 1990).

Also, there are significant differences in the distribution and thickness between fibers of outer membrane and inner membrane. The fibers distribute evenly in inner membrane and stretch out directly without branch. Fibers in outer membrane distribute unevenly, forming a disorder network. They go curvily and stretch out many branches. They often aggregate into lump. Such an uneven distribution of fibers in outer membrane may be caused by the influence of their contacting with papillar. The thick-

ness of fibers is not regular, but the diameter of fibers in outer membrane(<10  $\mu\text{m}$ ) is thicker than that in inner membrane(<3  $\mu\text{m}$ ). Beneath the inner membrane, a very thin membrane is found (Fig.1g) on which there are some chinks which are the passages of air. But these chinks are smaller than that of *Tetrastes sewerzowi* and *Crossoptilon auritum* (Liu Naifa 1992).

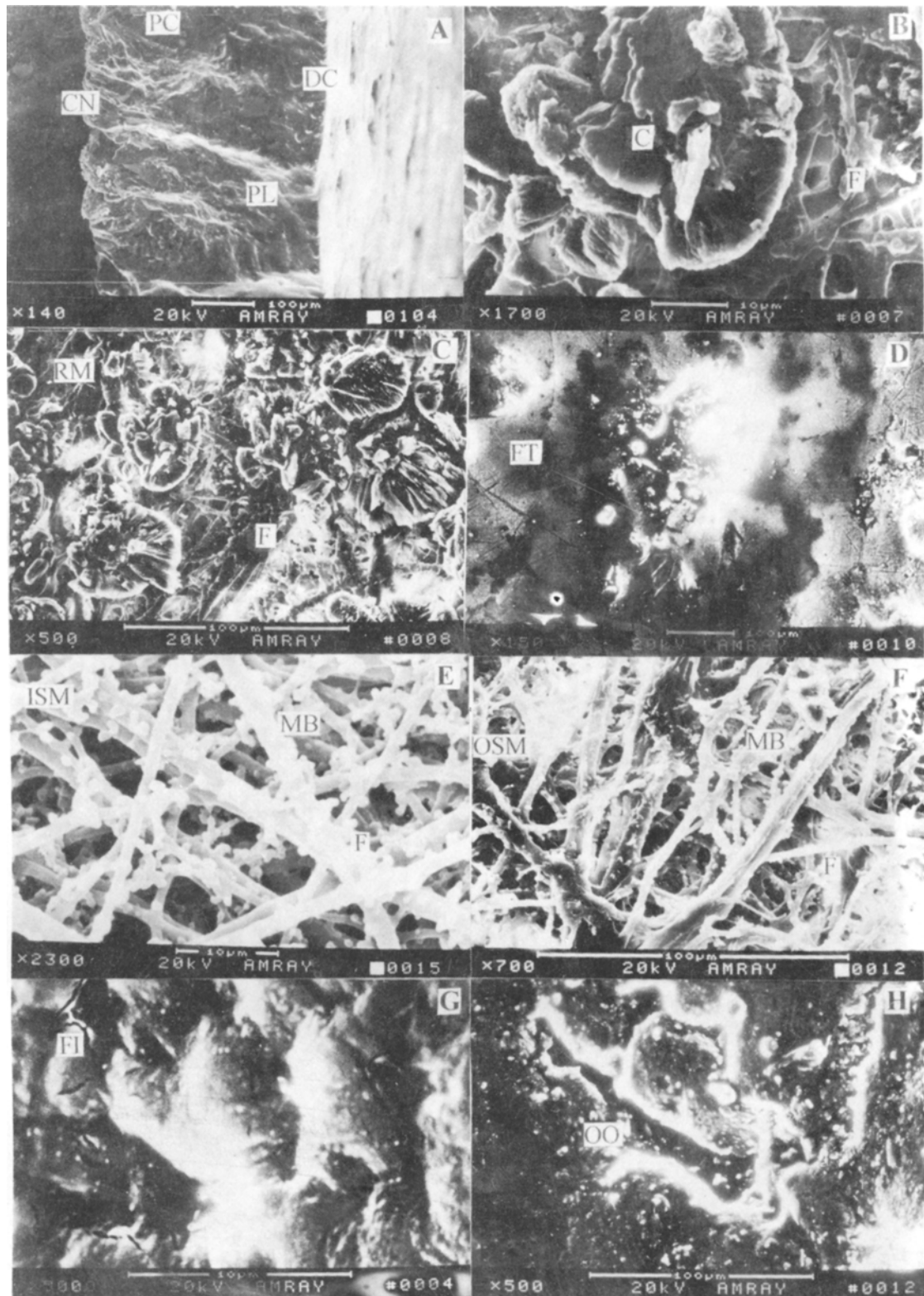
**Spiracle** Spiracle of great bustard are shaped like a flying bird(Fig. 8), which are different from that of other birds, such as elliptic spiracle in *Chrysolophus pictus*(Lu Taichun, 1992), lunise spiracle in *Crossoptilon mantchuricum*(Gan Yaling *et al.*, 1992).

Generally, eggshell structure of great bustard has common features of birds eggshell, such as structure of different layers, inner and outer membrane formed with protein fiber network, chinks on inner surface of inner membrane through which air can be ventilated. However, great bustard has some special characters of its own(Liu Naifa, 1994), for example, a smooth surface of eggshell unlike the moire crakes in poultry eggshell and spiracle pattern is like bird. Furthermore, there are some distinctions between outer membrane and inner membrane: the fibers of inner membrane are delicate netted structure without branches, but those of outer membrane are coarser and having branches. Some pellets that are supposed to be microbe parasiting on fibers. All these results will provide information for further knowing about the eggshell structure and for studying the physiology of incubation.

### Composition analysis of eggshell and feather

**Eggshell** The results of element analysis for eggshell of great bustard were shown in Table 1. Data of crested ibes(Li Fulai, 1990) and poultry and limitation of poisonous element content of World Hygiene Organization are also included. On the basis of the elements classification standard used in poultry farming, the 14 elements were divided into three kinds: general elements, trace elements and poisonous elements.

The content of calcium of great bustard eggshell(37.5%) was higher than that of crested ibes (32.7%) and poultry (36.4%); Magnesium content(0.18%) was higher than that of crested ibes (0.123%) but lower than that of poultry (0.389%); Sodium and Phosphorus contents of were 0.122%, 0.014% respectively, which are lower than those of crested ibes (0.179%, 0.13%) and poultry (0.152%, 0.116%). When the content of Calcium and Phosphorus were conversed to calcium carbonate and calcium phosphate, the contents of these two chemical compounds were 93.05% and 0.07% respectively. The calcium carbonate was significantly higher than those of crested ibes (81.23%) and poultry (90.2%),



**Fig. 1. Eggshell structure of great crested shown by scanning electron microscopic**

A.  $\times 140$  cross section of eggshell, showing papilar layer (CN), railings layer(PL) and air duct (PC); B.  $\times 1700$  cross section of papilar, showing papillar layer (C), outer membrasne fibers(F); C.  $\times 500$  cross section of papilar layer, showing outeer membran fibers (F), space among papi-lars(RM); D.  $\times 500$  out surface of eggshell, showing striaes(FT); E.  $\times 2300$  inner membrane of eggshell, showing inner membrane(ISM), inner membrane fiber(F) and parasiting microbe(MB); F.  $\times 700$  outer membrane of eggshell, showing outer membrane (OSM), outer membrane fiber (F) and parasiting microbe; G.  $\times 5000$  thin membrane of inner membrane, showing inner membrane(ISM) and chinks in inner surface (FI); H.  $\times 500$  spiracle, showing bird-shaped spiracle(OO)

while the content of calcium phosphate was significantly lower than those of crested ibis (0.65%) and poultry (0.58%). Trace element content excelled greatly that of crested ibis, especially the Copper content (4.0 ppm) was 50 times as much as that of crested ibis. Among poisonous elements except Strontium (<0.08 ppm) not having control group, Aluminium (0.4 ppm), Mercury (0.004 ppm), arsenic (0.108 ppm), Cadmium (0.04 ppm), respectively, were 4 times, 4 times, 2 times and 4 times as much as the pollution quota set by World Hygiene Organization. But these five poisonous elements except

**Table 1. Mineral analysis of eggshell of great bustard**

	General elements (%)				Trace elements (ppm)					Poisonous elements (ppm)				
	Ca	Mg	Na	P	Fe	Mn	Cu	Zn	Co	Sr	Pb	Hg	As	Cd
eggshell of great bustard	37.55	0.18	0.122	0.014	170.0	12.8	4.0	25.6	0.2	170.0	0.4	0.004	0.108	<0.04
eggshell of crested ibis*	32.78	0.123	0.179	0.13	128.7	5.33	<0.08	12.0	<0.1	233.2	<1.4	0.01	0.02	<0.05
eggshell of poultry	36.40	0.389	0.152	0.116										
Limited quantity set by world Hygiene Organization											0.1	0.001	0.05	0.01

\*from Li Fulai 1990

**Feather** Based on the Table 2, 14 elements content of female great bustard are higher than those of males, especially the content of five elements, Calcium, Manganese, Copper, Strontium and Mercury are different significantly between females and males. Content of Copper ( $\delta$  2.2 ppm,  $\eta$  6.6 ppm) and Cobalt ( $\delta$  <0.01,  $\eta$  <0.01) are lower than those of red crown crane (14 ppm, 1 ppm). However, content of Iron ( $\delta$  360 ppm,  $\eta$  365 ppm), Manganese ( $\delta$  8.7 ppm,  $\eta$  26 ppm), zinc ( $\delta$  200 ppm,  $\eta$  214

Arsenic were lower than those of crested ibis (Table 1). All these indicate that great bustard suffers from less pollution, compared to crested ibis.

The element content of eggshell is affected by its metabolism, and metabolism is influenced by the food and environment. So there are distinction among different elements content in eggshell. Through comparing content of poisonous elements, we can obtain an objective knowledge on the influence of food and environment on which animals depend. It benefits improvement of environment and successful breeding of population.

ppm) are all higher. In feather of great bustard, the content of poisonous elements such as Lead ( $\delta$  <0.05 ppm,  $\eta$  <0.05 ppm), Cadmium ( $\delta$  <0.01 ppm,  $\eta$  <0.01 ppm) are lower than those of red crown crane (3.7 ppm, 0.17 ppm). (The other three elements content are absent from reference). It suggests that wild great bustard suffers less pollution than captivity red crown crane does. This may be related to the numerous contaminated fish fed to captivity red crown crane.

**Table 2 Mineral analysis of great bustard's feather**

Species	General elements (%)				Trace elements (ppm)					Poisonous elements (ppm)				
	Ca	Mg	Na	P	Fe	Mn	Cu	Zn	Co	Sr	Pb	Hg	As	Cd
Great bustard $\delta$	4.50	0.06	0.07	0.009	360.0	8.7	2.2	200.0	<0.01	6.7	<0.05	0.055	0.337	<0.01
Great bustard $\eta$	11.19	0.07	0.08	0.011	365.0	26.0	6.6	214	<0.01	11.0	<0.05	0.139	0.566	<0.01
Red crown crane*					15	3.5	14	57	1		3.7			0.17

\*from Qin Zaixian 1986 (feathers on the chest of captivity red crown crane)

The composition content of eggshell and feather indicates that great bustard exhibits a normal physiological process, while red crown crane suffers from more disturbance by people. Poisonous element content of the former is significantly lower than that of the later. Therefore, the result of artificial raising will increase the poisonous element content in body. When they are raised artificially, attentions should be paid to preventing them from accumulating poisonous elements which will result in some faculty degeneration and disease.

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